PROGRESS REPORT No. 2

April 1, 1972-
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COLLAGEN ANTIBODIES IN RELATION TO THE ETIOLOGY OF EMPHYSEMA

During the period covered in this progress report, research the following problems have been carried out.

I. A detailed study on the ultrastructure of collagen fibers in lung has been completed.

II. A model system for emphysema induced in experimental animals has been developed in our laboratories, and ultrastructural alteration of lung collagen in these models is currently being studied.

III. The reactivity of chemicals present in cigarette smoke with lung collagen is currently being studied.

IV. A method for the extraction and purification of lung collagen has been devised in our laboratories allowing detailed chemical analysis of amino acids and carbohydrate components of this collagen.

V. A study on the immunological characteristics of normal lung collagen and of lung collagen reacted with chemicals present in cigarette smoke has been initiated.

VI. A survey of anti-collagen titers in emphysema patients and the normal population has been continued and expanded.

DETAILED SUMMARY REPORT

I. The freeze fracture technique was used to study the fine structure of collagen from rat tendon and lung. The collagen filaments were organized into a spiraled lamellar substructure within each fibril. Filamentous connections were shown which span the inter-fibriller matrix and unite all of the fibrils into a reticular network. These connections had a range in diameter between 70 to 100 Å. The fibrils were coated with material that was greatly hydrated *in vivo*. On the basis of these observations, we have proposed a new structural model for the collagen fibril. A manuscript has been submitted to the Journal of Ultrastructural Research (1), and another is in preparation.

II. We have observed that chronic administration of high doses of β aminopropionitrile induces changes in the lung of experimental animals that are histologically similar to the changes observed in emphysema. These included breakdown of alveolar septae, atrophy of capillaries and general loss of elasticity of the lung. We are currently engaged in

examining these changes on the ultrastructural level. We are also studying the immune response of these animals to their lung components, especially lung collagen.

III. Studies on the interaction between acetaldehyde and lung collagen have been initiated. Preliminary results indicate that ε amino groups of lysine residue in the collagen molecule interact with this chemical. We are planning to use radioactive acetaldehyde to determine:

a) The quantitative aspects of this interaction.

b) The location on the molecule of acetaldehyde binding.

c) To determine whether acetaldehyde becomes and antigenic determinant following its interaction with collagen.

We are also planning to examine the ultrastructural effects of acetaldehyde binding to collagen.

IV. Although minute amounts of collagen could be obtained from lung using the classical methods of extraction, we could not obtain sufficient material for detailed chemical and immunological studies. Therefore, it was essential to develop an extraction technique that will increase the yield of this collagen. We found that limited digestion with trypsin at 10°C for 24 hours resulted in a substantial increase in the solubility of lung collagen. This collagen is currently being subjected to the following analysis:

a) Chromatography on CM cellulose columns to separate the polypeptide chains of collagen.

b) CNBr digestion of the isolated chains and chromatography on phosphocellulose and CM cellulose columns. This will allow the rapid determination of any sequence differences between lung collagen and skin-type collagen.

c) The content and composition of the carbohydrate moiety of this collagen is being determined. Also, the availability of large amounts of lung collagen has enabled us to inject rabbits with this collagen at high enough doses to obtain high titers of antibodies.

V. In addition to the above mentioned immunization with normal collagen, we are in the process of preparing large enough quantitites of collagen reacted with acetaldehyde in order to determine whether such an interaction induced immunological changes in the collagen. Specifically, we will test for new determinants which are an integral part of the collagen molecule as well as the possibility that

acetaldehyde constitutes a major new determinant.

VI. A substantial sample of emphysema patients and normal population has been assayed for anti-collagen antibodies. The results indicate significant increased incidence and titer of anti-collagen in emphysema patients. The results are currently being statistically evaluated and will be published shortly (2).

VII. Methods to test the cellular response of emphysema patients to a specific antigenic determinant to human lung collagen by in vitro addition of the small peptide possessing highest antigenic activity to lymphocytes of patients with emphysema in vitro are awaiting isolation of the small peptide

VIII. In terms of macrophages an animal model for macrophage activity has been developed depending upon the production of a microsomal enzyme, hemoxygenase, by macrophages after ingestion of antigen antibody complexes. The rate of production of the enzyme has the characteristics of an induced enzyme and is dissociated from phagocytosis in that there is a lag phase, a hydrocortisone supressed enzyme induction but this inhibitory effect could be reversed by the addition of glucose and insulin to a culture medium. The initial findings were done with macrophages obtained from rat peritoneal cavity, but similar findings have been obtained with rabbit alveolar macrophages (3).

IX. In preparation for looking at pulmonary alveolar tissue by freeze etch electron mechanism, pulmonary studies using the technique were performed with erythrocyte membrane. One published paper demonstrated the translational movement along the the plane of the human erythrocyte ghost of the membrane particles exposed by freeze-fracture. The membrane particles can be aggregated by incubation of the ghosts in media with a pH in the vicinity of 5.5 or 3.5. The particles are disaggregated in neutral and alkaline media (pH 9.5) and also at pH 4.5. Aggregation of the particles is reversible, prevented by prefixation in glutaraldehyde and by media of high ionic strength. Particle aggregation occurs within two to four minutes. These results are consistent with the concept that the erythrocyte ghost membrane is a planar fluid domain formed by a bilayer membrane continuum which is interrupted by localized, yet mobile, proteic intercalations (4).

REFERENCES

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